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A NEW HEMICHORDATE, *SACCOGLOSSUS BROMOPHENOLOSUS* (HEMICHORDATA: ENTEROPNEUSTA: HARRIMANIIDAE), FROM NORTH AMERICA

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Abstract.—A new species, *S. bromophenolosus,* is distinguished from its congener, *S. kowalevskii* (Agassiz 1873), on the basis of the following morphological, biochemical, and genetic criteria: placement of gill pores; prominence of the dorsal ridge; structure of the proboscis skeleton; presence of bromophenols or bromopyrroles; relative electrophoretic mobility of allozymes (e.g., superoxide dismutase); molecular weights of fragments from restriction endonuclease digestion of mitochondrial DNA. *S. bromophenolosus* occurs from southern Maine, U.S.A., to Nova Scotia, Canada, with an additional record from Willapa Bay, Washington, U.S.A.

Three families of enteropneusts (Hemichordata: Enteropneusta: Harrimaniidae, Ptychoderidae, and Spengeliidae) occur along the coasts of North America (Milne & Milne 1973, Bullock 1975, Ruppert & Fox 1988). The best known species belong to the genus *Saccoglossus* (Harrimaniidae), which occurs on both the Atlantic and Pacific coasts (Milne & Milne 1973, Bullock 1975). Of the several saccoglossids, *S. kowalevskii* (Agassiz 1873) has been described in greatest detail. Various aspects of its distribution, biology and ecology have been reported subsequent to Agassiz's (1873) initial description (e.g., Bateson 1886, Bullock 1940, Tweedell 1961, Colwin & Colwin 1962, Barrington 1965, King 1986, Woodin et al. 1987, Balser & Ruppert 1990, Carey & Mayer 1990). *S. kowalevskii* has been considered the only member of its genus on the Atlantic coast of North America, and has been noted in intertidal collections from Nova Scotia to Georgia (Dörjes 1972, Bromley 1979). Although there are certain characteristics common to all saccoglossids along this range, a comparison of biochemical attributes reported for populations from Maine and South Carolina has raised questions about the taxonomic status of the species (King 1986, Woodin et al. 1987). Specifically, animals collected in Maine and northward typically accumulate high concentrations of two secondary products, 2,4-dibromophenol and a dibromoindole (King 1986, unpubl. data), while animals from other locales accumulate 2,3,4-tribromopyrrole (King, unpubl. data, Woodin et al. 1987). The differential accumulation of these haloaromatics does not appear to correlate with any major environmental variables or gradients. Further, the presence of a given haloaromatic phenotype appears to be a fixed trait associated with populations from specific regions.

We now report that the genus *Saccoglossus* consists of at least two distinct species along the Atlantic coast of North America. These two species are readily differentiated by gross external morphological characters, the accumulation of halogenated aromatic compounds, morphology of the proboscis skeleton, electrophoretic mobilities of several enzymes, and the sequence composition of the mitochondrial DNAs (mtDNA). We propose the name *S. bromophenolosus* for the new species occurring from southern

Maine northward and possessing features as detailed below. *S. kowalevskii* occurs from southern Maine southward, as noted by Verrill (1873) and Gosner (1979).

Materials and Methods

Saccoglossids were collected from the intertidal zones of sites ranging from Halifax, Nova Scotia, to Georgetown, South Carolina, by excavating sediments to a depth of about 10 cm at low tide. The proboscis, collar and anterior portions of the trunk were obtained readily by removing the animals directly from the substrate, but complete specimens were difficult to collect due to the fragility of the posterior region of the trunk. Specimens were examined in the field for external morphological characteristics (see below) and then placed individually in small vials (20 ml) containing local seawater; the animals were subsequently transported to Walpole, Maine, for further processing. MtDNA was extracted from live animals using procedures modified from Lansman et al. (1981) within 24–48 h of return to the laboratory. A minimum of 10 live animals from Lowes Cove and York, Maine (69°34′N, 43°56′W; 43°09′N, 70°39′W) and from Portsmouth, New Hampshire (43°06′N, 70°42′W) were also homogenized for electrophoretic analyses of enzymes at the following loci using standard methods (Murphy et al. 1990): glucose phosphate isomerase (GPI; E.C. 5.3.1.9), malate dehydrogenase (MDH; E.C. 1.1.1.37), phosphoglucomutase (PGM; E.C. 2.7.5.1), and superoxide dismutase (SOD; E.C. 1.15.1.1). Analyses of haloorganic contents utilized extracts of animals that were extracted with hexane immediately after collection and removal of adhering sediment. In addition, animals used for mtDNA and allozyme analyses were "halotyped" by collecting 1–2-μl samples of the seawater immediately adjacent to the proboscis with a 10-μl gas chromatography syringe (Hamilton Inc., Reno, Nevada). The epidermis of the proboscides was irritated with the syringe needle prior to sample collection in order to stimulate mucus and haloorganic excretion. The samples were analyzed by direct injection into a gas chromatograph. Details of the extraction and analytical procedures have been reported elsewhere (King 1986, 1988). The proboscideal skeletons of 5 individuals from Lowes Cove and York, Maine, were examined after dissection of live animals.

Saccoglossus bromophenolosus, new species

Figs. 1–4, Table 1

Saccoglossus sp. Bullock 1975, p. 619; Kozloff 1987, p. 478.

Saccoglossus kowalevskii.—Milne & Milne 1973, (p.p.): 230; Bromley 1979, p. 533.

Saccoglossus kowalewskyi.—Brinkhurst et al., 1976, p. 156.

Saccoglossus kowalevskyi.—King, 1986, p. 257.

Saccoglossus kowalewskii.—Linkletter et al. 1977, (p.p.): 42, Gosner 1979, p. 265; Meinkoth, 1981, (p.p.): 726; Carey & Mayer, 1990, p. 79.

Diagnosis.—*Saccoglossus bromophenolosus* attains a length up to 20 cm. It has an elongate proboscis that extends 1.5–2 cm in narcotized specimens. The proboscis has a shallow dorsal groove and a single pore at the base of the proboscis. Concretions fill the primary shaft of the proboscis skeleton. Hexane extracts of the proboscis contain 2,4-dibromophenol and a dibromoindole; these compounds account for the characteristic "bromoform" odor of live specimens. The collar is differentiated into 4–5 zones, and is generally rectangular from above with an aspect ratio of about 3:5 (width : length). The trunk is differentiated into distinct branchial and hepatic-genital regions. Dorsolaterally placed gill pores appear ellipsoid in relaxed and ventilating specimens, with the major axis oriented vertically; the gill pores do not occur in folds and vary from about

60–110 pair per individual. Esophageal pores, which occur slightly posterior to the gill pores vary from about 4–8 pair in number. Gonads begin at the terminus of the branchial region in both sexes, and are dorsolateral in placement.

Material examined.—Holotype (USNM 168049) and 3 paratypes (USNM 168050-168052) from Lowes Cove, Maine (69°34'N, 43°56'W), collected by G.M. King 20 October, 1993.

Description.—The holotype is a sexually immature female with the following characteristics observed while the animal was relaxed in a solution of 7% $MgCl_2$. The incomplete specimen was 82 mm in length; an additional 60–70 mm of the posterior-most region of the trunk was lost unavoidably during collection. The posterior-most section of the trunk was extremely thin and fragile; in color and diameter, it resembled the fecal coils present at the sediment surface. The relaxed and extended proboscis was creamy white in color with a shallow dorsal groove running from the base to the proboscis tip. Striations perpendicular to the major axis of the proboscis were observed at low power on a dissecting microscope. The proboscis was 16 mm in length and 3 mm at the base. The basal-most region was a rust orange in color. The orange collar was 5 mm in length and 2.5 and 3 mm in diameter at the anterior and posterior ends, respectively. Both ends of the collar were distinctly thickened, with a prominent lighter-colored ridge circling the posterior of the collar. The branchial region of the trunk was about 2.5 mm in diameter where it joined the posterior of the collar. A prominent, raised dorsal ridge began immediately posterior to the collar and ran the length of the branchial region, terminating just anterior to the esophageal pores. Near the collar, the dorsal ridge was about 1 mm in width with a central groove about 0.5 mm in width. This region of the trunk was pinkish-orange to orange and about 14 mm in length. A total of 61 pairs of gill pores were located dorsolaterally, beginning at the junction of collar and trunk. Slightly posterior to the terminal gill pores, 4 pairs of dorsal esophageal pores were observed; they formed an angle of about 30° opening toward the trunk. The hepatic-genital region of the trunk extended about 23 mm, measured from the termination of the gill pores to a point where genital structures were not observable. Nascent grayish egg masses were visible dorsolaterally just beneath the epidermis. The hepatic-genital region was grayish-brown dorsally and yellowish-brown laterally. The posterior-most region of the trunk was 34 mm in length, yellowish-brown in color and characterized by pairs of carmine-colored spots located dorsally.

General dimensions for 3 male paratypes with maturing gonads were similar to those of the holotype. However, gill pore numbers ranged between 68–101 and esophageal pores numbered 4–6. The sperm sacs were dorsolateral and pink in color.

Remarks.—Although the range of body size, coloration, location and appearance of the gonads, and external features of the collar and proboscis of *S. bromophenolosus* and *S. kowalevskii* are very similar, gill pore placement and the morphology of the dorsal ridge allow discrimination of the species by visual inspection in the laboratory or field (Fig. 1, 2). In contrast to *S. bromophenolosus,* the dorsal ridge of *S. kowalevskii* is relatively broad and flattened immediately posterior to the collar, covering >50% of the dorsal area. The dorsolateral gill pores of *S. kowalevskii* occur within lateral grooves that can be closed, thereby obscuring them from view (Fig. 2). This state is typical of specimens preserved without prior relaxation. In addition to external morphology, *S. bromophenolosus* and *S. kowalevskii* can be distinguished by other diagnostic characteristics requiring dissection or various laboratory analyses (Fig. 3, Table 1). Collections to date indicate that *S. kowalevskii* and *S. bromophenolosus* exist sympatrically only within a narrow range around the mouth of

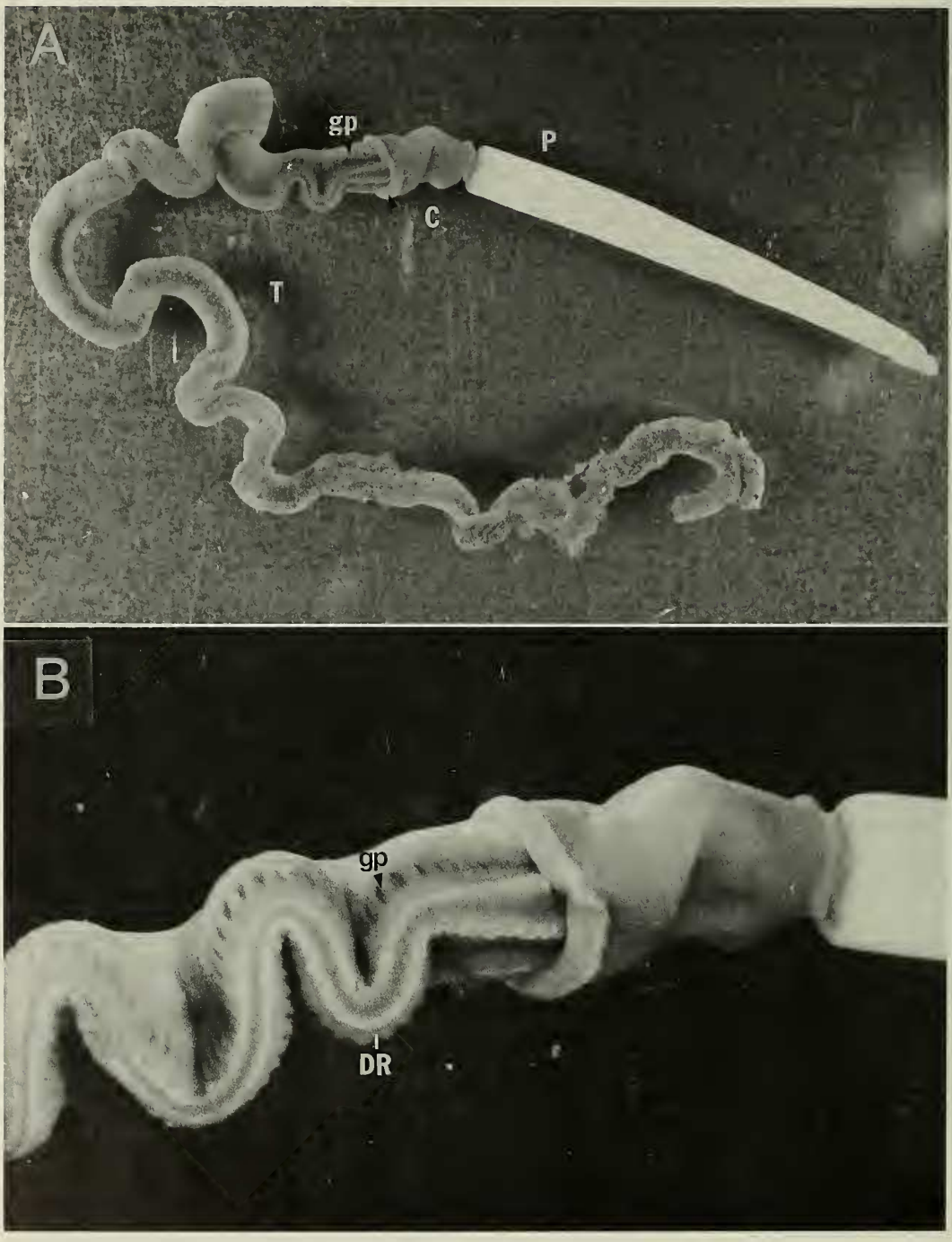


Fig. 1. A. Dorsolateral view of relaxed, sexually immature female of *S. bromophenolosus,* new species illustrating superficial features of the proboscis (P), collar (C, limits indicated by arrows) and trunk (T); gill pores (gp, indicated by arrow) are evident immediately posterior to the collar. B. Detailed view of the posterior proboscis, collar, and anterior of the trunk, illustrating the prominent, raised dorsal ridge (DR) and gill pores.

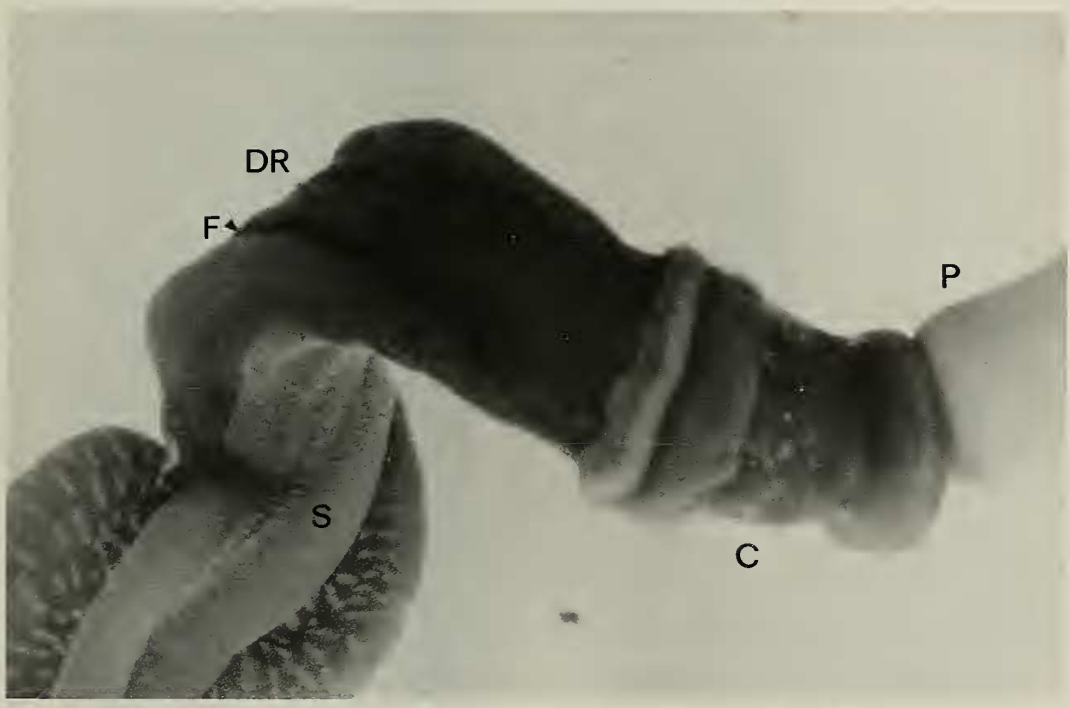


Fig. 2. Anterior portion of *S. kowalevskii* showing the broad, flattened dorsal ridge (DR), collar (C) and proboscis (P); gill pores are obscured by lateral folds (F, indicated by arrow). The "sole" (S) occurs ventrally.

the Piscataqua River, New Hampshire (43°06′N, 70°42′W); *S. kowalevskii* has not been observed north of York, Maine, while *S. bromophenolosus* has not been found south of Portsmouth, New Hampshire.

Allozyme analyses indicated substantial divergence between *S. bromophenolosus* and *S. kowalevskii* since GPI, MDH, and PGM were fixed for alternative, diagnostic alleles within each taxon; SOD was represented at 2 monomorphic loci in *S. bromophenolosus*, and by a single, different monomorphic locus in *S. kowalevskii*. MtDNA analyses also revealed substantial divergence (Fig. 4).

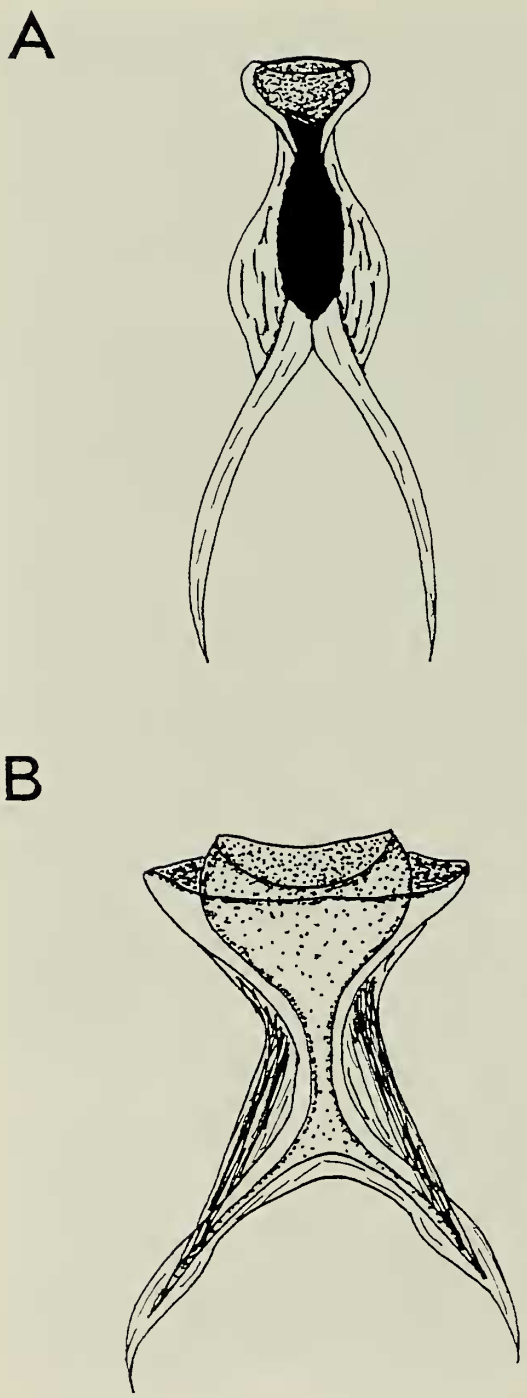
Table 1.—Diagnostic characters that distinguish among 3 species of North Atlantic saccoglossids.

	S. bromophenolosus	*S. kowalevskii*	*S. ruber*
Characteristic			
Dorsal ridge	raised, narrow	broad, flattened	raised, narrow
Gill pore placement	central	lateral	central
Coloration[1]			
(proboscis-collar-trunk)	W/P-O/R-O	W/P-O/R-O	P/R/R-O
Halotype[2]	DBP/DBI	TBPy	DBP/TBP
Proboscis skeleton[3]	narrow curve-co	broad curve-no co	narrow curve-co

[1] Color code: W/R, ranges from white to pale pink; O/R, ranges from orange to red; O, orange; P/R, pale pink to pale red.

[2] Compound code: DBP = 2,4-dibromophenol; DBI = dibromoindole (positions of bromine atoms uncertain); TBP = 2,4,6-tribromophenol; TBPy = 2,3,4-tribromopyrrole.

[3] Co refers to concretions within the proboscis skeleton.



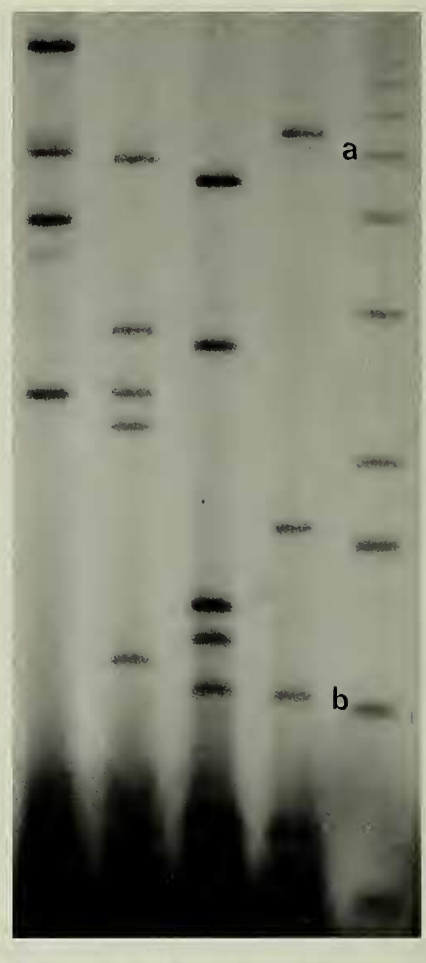


Fig. 4. Autoradiogram of fragments from the digestion of pooled mtDNA from specimens of *S. bromophenolosus*, new species (lanes 1, 3) or *S. kowalevskii* (lanes 2, 4) with the restriction endonucleases *Hind* III (lanes 1, 2) or *Sty* I (lanes 3, 4). Fragments were separated after digestion by agarose gel electrophoresis and radiolabelled with ^{35}S using a nick translation procedure. Lane 5 contains a molecular weight standard (1 kb ladder, BRL); "a" and "b" indicate 5090 and 1018 base pair fragments, respectively.

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Fig. 3. A. Diagram of the proboscideal skeleton of *S. bromophenolosus*, new species; dark central region consists of concretions as described for *S. ruber* (Burdon-Jones & Patil 1960). B. Diagram of the proboscis skeleton of *S. kowalevskii*; note absence of central concretions and more deeply curved skeletal arms.

No common DNA fragments were found in comparisons of restriction endonuclease digests of the mtDNA of the two taxa based on *Hind* III, *Xba* I, *Nde* I, *Sty* I, *Stu* I, *Dra* I or *Ava* I. In contrast, mtDNA haplotypes for populations of each taxon were homogenous. For example, an *Xba* I digest yielded the following approximate fragment molecular weights: *S. bromophenolosus*—8750, 1075, 925, and 625, *S. kowalevskii*—5000, 3225, 2275, 1875, and 1000.

Etymology.—The species name is derived from its characteristic haloorganic content, 2,4-dibromophenol, and the Latin suffix,-*osus*; thus *S. bromophenolosus, Saccoglossus* "with bromophenol."

Distribution.—On the east coast of North America, the range of *S. bromophenolosus* extends north from the mouth of the Piscataqua River separating Maine and New Hampshire to at least Halifax, Nova Scotia. *S. bromophenolosus* is found in silty sands throughout the intertidal zone in this range. It is often distributed in patches, with densities from about 10–>100 individuals m^{-2}. In addition, a sub-tidal form has been recorded from the Damariscotta River (69°34′N, 43°56′W) at a depth of 10–20 m. Specimens obtained from Willapa Bay, Washington (46°37′N, 124°00′W) have very similar mtDNA haplotypes and 16S ribosomal DNA sequences (pers. observations); in addition, the external morphologies and haloorganic contents of these organisms are indistinguishable from *S. bromophenolosus* (K. Woodwick, in litt.). Since these northwestern Pacific saccoglossids represent a previously unnamed species (Kozloff 1987), we incorporate them as trans-Arctic representatives of *S. bromophenolosus*. The range of the western North American populations is uncertain, but appears to include Oregon and Washington (Bullock 1975, Kozloff 1987).

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